X	**Experiment title:** A SAXS study of the giant TET protease from hyperthermophilic and barophilic *Pyrococcus horikoshii*	**Experiment number**: SC-2189
Beamline: ID02	**Date of experiment**: from: 18-MAY-2007 to: 21-MAY-2007	**Date of report**: 31-AUGUST-2007
Shifts: 9	**Local contact(s)**: Dr Emanuela DI COLA	*Received at ESRF:*

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Report:

We were granted three days of beamtime at ID02 (SC-2189 "A SAXS study of the giant TET protease from hyperthermophilic and barophilic *Pyrococcus horikoshii*") to study a protein complex with a pressure cell (developed by Dr. Stéphanie Finet). During the first two days we obtained promising results from the protein complex at low concentration (1 mg/ml, chosen for biochemical reasons).

Data on the protein solution and the buffer (for background substraction) were measured under several conditions. The first series was done on a more concentrated sample (5mg/ml). The aim in this series was to test the radiation resistance of the protein and to check if there were any problems with the temperature and pressure system. The protein suffered no detectable radiation damage, so the protein concentration was reduced to 1mg/ml and another test of radiation resistance was done. Samples were then measured at pH 7 and 9 at temperatures of 20, 60 and 90°C and pressure varying from 0-300MPa in steps of 50MPa. Additionally we tested the influence of EDTA under the same conditions of temperature and pressure at pH 7.

Our data clearly show that the protein maintains its quaternary structure even at 300MPa and 100°C. To our knowledge no other protein complex of comparable size has been discovered that can resist such extreme conditions. A manuscript combining our SAXS results with those of biochemical experiments under high pressure is in preparation.

Unfortunately, in contrast to usual capillaries, the pressure cell produces a high parasitic background at very small angles (see Fig. 1). Due to the size of our protein complex (R_g = 50Å), this parasitic background makes a Guinier analysis difficult in our case. Therefore, we are using the position of the first minimum and first

side maximum as parameters for the structural analysis of the overall shape of the protein complex. This method yields better results when applies to data measured at a protein concentration of 5mg/ml. We had foreseen to measure the corresponding data sets also at a higher concentration (5 mg/ml) on the third day. Unfortunately, we lost the third day due to mechanical problems with the pressure cell.

To improve data quality by measuring on a more concentrated sample of TET3 and in order to do the same kind of measurements on other TET-like proteins, we will apply for more beamtime on ID02.

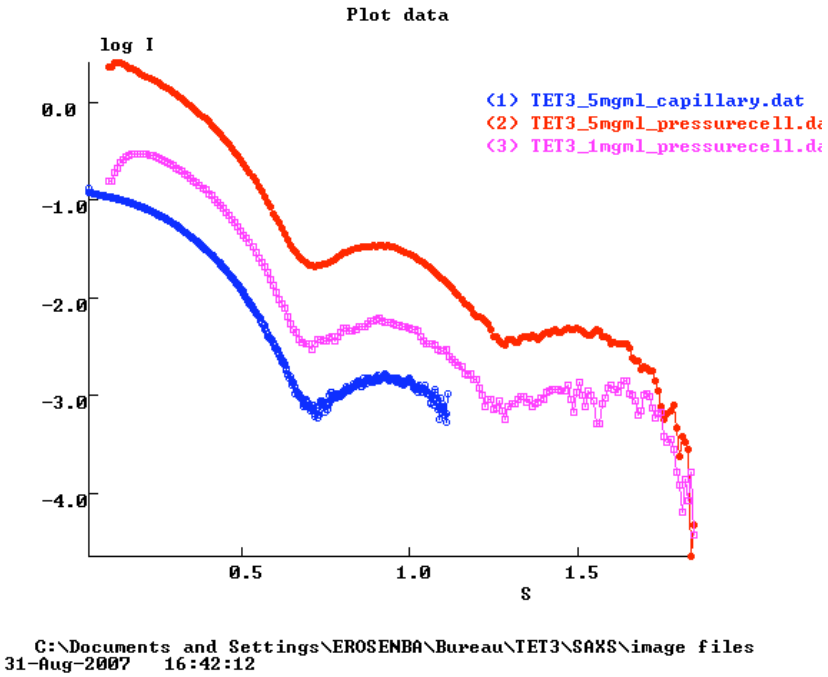

Figure 1: SAXS curves comparing the protein complex TET at different concentrations measured in a capillary and in the pressure cell. Curves (2) and (3), measured in the pressure cell, clearly show artifacts at small S-values due to parasitic scattering. The first minimum and the first side maximum are reasonably well defined in all three curves.